3.2 Optimization of UAE conditions

3.2.1. Selection of solvents in UAE

The selection of the most appropriate solvent for extracting bioactive compounds from the sample matrix is an important process for investigating any extraction techniques[20]. Here, water, ethanol, methanol and ethyl acetate were selected as the extracting solvents. We tested the effects of different solvent under the same condition: sample of 0.5 g, solvent to solid ratio of 10 mL/g, temperature of 30℃ and extraction time of 30 min. The results indicated that the highest total extraction yields of the three phenylpropanoids was obtained by methanol and the lowest yield was obtained by ethyl acetate.The total extraction yields of the analytes were methanol (1.46 mg/g) > water (1.12 mg/g) > ethanol (0.84 mg/g) > ethyl acetate (0.31 mg/g). So we finally selected the methanol as the extract solvent.

3.2.2. Effects of extraction solvent concentration

In order to investigate the effect of methanol concentration on the phenylpropanoids yield, various methanol concentrations were tested, and the results were shown in Fig.3A. Methanol-water mixtures were found to be more effective for the extraction of syringin, clemastanin B and indigoticoside A than absolute methanol or water. The increase in the extraction yields of the three phenylpropanoids with the introduction of water to methanol may be attributed to the increase in permeability of plant issues in the presence of water in extracting solvents, which enables better mass transfer by diffusion.

The results shown in Fig.3A clearly indicated that the highest extraction yields of

syringin, clemastanin B and indigoticoside A were obtained by water/methanol (20:80 v/v).Thus, 80% methanol was chosen as the best solvent in the following extraction experiments.

3.2.3. Effect of ultrasonic time

To investigate the influence of extraction time on yield of phenylpropanoids, 0.5 g sample was extracted at the conditions of 30℃ and 5 mL of water/methanol (20:80,v/v) under the different ultrasonic time (10, 20, 30, 45, 60 ,90 min).The results showed in Fig.3B clearly indicated that the extraction yields of syringin, clemastanin B and indigoticoside A increased with the increase of extraction time from 0 to 45 min. However, the extraction yields have no significant change between 45 and 90 min ,suggesting the extraction efficiency has maximized. As the extraction time prolonged, most of the plant cells will be completely cracked because of acoustic cavitation effects, and extraction yield increases. However, when the plant cells rupture, various compounds such as insoluble substances and cytosol suspend in the extraction liquid, thus resulting in the lower permeability of the solvent[21]. Also, the objective constituents will re-adsorb on the smashed plant particles due to their relatively large specific surface areas and decrease yields of recovered compounds[22].Hence, 45 min was chosen as the optimum extraction time.

3.2.4. Effect of the ratio of liquid/solid

The solvent quantity is also important factor to the yield of extraction[23]. Therefore, we determined its influence on the extraction yield to obtain optimum ratio of liquid/solid.Here, we investigated the effect of the ratio of liquid/solid (5, 10, 15,

20, 30, 40 mL/g) on the extracting yields of syringin, clemastanin B and indigoticoside A at 30℃ for 45 min. Data shown in Fig.3C indicated an obvious increase of extraction yields of syringin, clemastanin B and indigoticoside A when the solvent to solid ratio was increased from 5 to 20 mL/g ,but there was no obvious change in the yields of syringin, clemastanin B and indigoticoside A as the ratio continued to increase. To avoid the wasting consumption of solvents, 20 mL/g was chosen as the optimum ratio of the solvent to solid.

3.2.5. Effect of temperature

In this study, six different temperatures (30, 40, 50, 60, 70 and 80℃) were selected to evaluate the influence of temperature on the extraction efficiency and quality of phenylpropanoids from Radix Isatidis with the optimum conditions fixed as mentioned in Section 3.1.3. As shown in Fig.3D, the extraction yields of the three phenylpropanoids increased with the increase of extraction temperature when the temperature ranged from 30 to 60℃, then the yields decreased with the temperature continuing to increase. Too high temperature had a negative effect because that high temperature led to the decrease of surface tension and the increase of vapour pressure within micro bubbles, causing the damping of the ultrasonic wave[24]. Based on these results, the optimum extraction temperature was 60℃.

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